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In Vitro Factor VIII Recovery During the Delivery of Ultra-Pure Factor VIII Concentrate by Continuous Infusion

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Factor VIII (FVIII) replacement by continuous infusion has been advocated as a cost-effective method for maintaining stable plasma levels of FVIII in the hemophilia A patient during surgery or life-threatening hemorrhage. Continuous delivery of monoclonal or recombinant FVIII concentrates to our pediatric patients using a traditional delivery system (dilution in normal saline of 2–10 U/ml infused at a rate of 20 ml/hr) has frequently yielded higher than expected factor usage to achieve desired levels and unexpected variability in plasma levels under presumed steady-state conditions. To determine if diminished in vitro FVIII recovery was responsible for these observations, a study of four ultrapure concentrates during 8 hr of in vitro continuous delivery was performed using four delivery systems. When reconstituted concentrate was added to normal saline in polyvinylchloride bags at a concentration of 10 U/ml (method IA), monoclonal products showed a stable recovery of 84–109% of time 0 levels. Recombinant product recovery dropped to 57–76% of time 0 levels before reapproximating the time 0 level at 2 hr. The addition of 10 mg/ml human albumin to the bags (method IB) did not improve recoveries. When reconstituted concentrate was delivered undiluted (method IIA), the early drop in recombinant recovery was eliminated; stable recovery of 78–117% of time 0 level was achieved with all products. In using method IA, a large discrepancy was seen between the actual time 0 recoveries and those expected based on vial assays, most striking for recombinant products (49–57% of expected). Method IIA allowed 75–90% recovery; addition of 20 mg/ml albumin of reconstituted but undiluted concentrate (method IIB) maximized recovery at 85–98% of expected.

Key words: factor VIII, hemophilia A, therapy, concentrates

INTRODUCTION

The administration of factor VIII (FVIII) replacement by continuous infusion (CI) was first shown in 1970 by McMillan et al. [1] to be an efficacious method for maintaining stable in vivo plasma FVIII activity levels in hemophilia A patients with life-threatening hemorrhage or undergoing major surgery. The efficacy and reliability of this method for the delivery of low- and high-purity FVIII was subsequently corroborated by other investigators [2–4]. The cost effectiveness of CI compared to conventional bolus injection was initially demonstrated by Hathaway et al. [2] and confirmed by Bona et al [3]. Martinowitz et al. [4] later attributed the approximate 30% reduction in average factor usage to a progressive decrease in FVIII clearance rate over the first 5 days of CI.

By the late 1980s and early 1990s, very high or ultrapure FVIII concentrates were in widespread use. Ultrapure product is generally defined by a specific activity of at least 3,000 U/mg protein prior to albumin stabilization and is produced by the monoclonal-antibody purification of either plasma-derived or recombinant FVIII [5]. Monoclonal-antibody purified concentrate delivered by CI was previously demonstrated to behave similarly to lower purity product with respect to clinical effectiveness, regardless of whether the factor was infused after simple reconstitution according to manufacturers' recommendations [6] or traditionally diluted in normal saline [7]. Although the recombinant FVIII products have not been comparably studied, the recovery of one such product was shown by Schulman et al. [8] to be stable for at least

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3 days when stored at room temperature in polypropylene after simple reconstitution. However, at our institution, attempts to deliver continuously monoclonal or recombinant FVIII concentrates to pediatric hemophilia A patients with actual or potential major hemorrhage has frequently yielded clinically unsatisfactory results. Use of a traditional delivery system in which reconstituted product is further diluted in normal saline to final concentrations between 2 and 10 U/ml and infused at a rate of 20 ml/hr resulted in 1) higher than expected factor usage to achieve desired plasma FVIII activity levels and 2) unexpected variability in plasma FVIII levels under presumed steady-state conditions. This study was therefore designed to determine whether diminished in vitro FVIII recovery in this system was responsible for the in vivo observations as well as to develop the optimal CI delivery system for ultrapure products in our hospital setting.

MATERIALS AND METHODS

Factor VIII Concentrates

FVIII concentrates studied included two plasma immuno-monoclonally purified products (Hemofil M [Baxter/Hyland] designated monoclonal 1 and Monoclate P [Armour] designated monoclonal 2) and two recombinant products (Kogenate [Bayer] designated recombinant 1 and Recombinate [Baxter/Hyland] designated recombinant 2). All products were generously donated by the manufacturers.

Factor VIII Concentrate Standard

FVIII concentrate standard MEGA-1, generously provided by W. Fricke at the Food and Drug Administration (FDA), was used to construct standard curves for all assays. The assigned value of the lot was 10.2 IU/vial. Each vial was reconstituted with 1 ml of distilled water as per manufacturer's instructions.

FVIII Assays

FVIII activity assays were performed on all samples and reconstituted vial contents as recommended by the Factor VIII/IX Subcommittee of the International Society for Thrombosis and Haemostasis [9]. A one-stage assay was performed using substrate plasma naturally deficient in FVIII (Helena Laboratories, Beaumont, TX) and veronal buffer (Dade/Baxter Healthcare, Miami, FL) containing 10 mg/ml bovine serum albumin (Sigma A3059; protease-free). All assays were performed on an optical instrument, the MLA Electra 900C (Medical Laboratory Automation, Inc., Pleasantville, NY). Standard curves were constructed daily by prediluting the MEGA-1 standard 1:10 in the FVIII-deficient plasma prior to further dilutions of 1:5 to 1:640 in the albumin-veronal buffer.

Continuous Infusion Delivery Systems

Delivery systems were established to approximate closely the conditions under which the concentrates would be prepared by the pharmacy and delivered to the patient by CI. All concentrates were initially reconstituted according to manufacturers' specifications with the diluent provided. The infusions were calculated to deliver a dose of 200 U/hr (4 U/kg/hr for a 50 kg individual) for 8 hr under four different sets of conditions. In method IA, reconstituted concentrate was added to normal saline (NS; 0.9% NaCl) in polyvinylchloride (PVC) bags to an approximate final concentration 10 U/ml based on manufacturers' assay of the vial contents. This mixture was delivered through PVC tubing at a constant rate of 20 ml/hr by IVAC pump (IVAC, San Diego, CA). Method IB was identical to method IA except that the bags also contained additional human albumin at a final concentration of 10 mg/ml. Only recombinant products were tested by method IB. In method IIA, reconstituted concentrate was delivered undiluted (approximately 100 U/ml by manufacturers' assays of vial contents) by 60 ml Becton-Dickinson polypropylene syringe using a Medfusion 2001 syringe pump (Medfusion Inc., Duluth, GA) through Medfusion Mini-vol 60 inch PVC tubing. A constant rate of 2.5 ml/hr was used. This infusion system is known to deliver with an accuracy of $\pm 10\%$, even at flow rates significantly lower than those used in this experiment [4]. Method IIB was identical to method IIA except that additional human albumin was added to the syringes for a final concentration of 20 mg/ml. Recombinant products were studied on two occasions with method IA, and those results are presented as averages. All other studies represent a single experiment.

Sample Collection and Preparation

Samples were collected into polypropylene tubes at times 0, 0.25, 0.5, and 1 through 8 hr by free flow from the distal end of the tubing, followed by immediate assay. For method IIB, only time 0 specimens were collected. Samples for methods IA and IB were diluted 1:10 in FVIII-deficient plasma prior to assay. Samples from methods IIA and IIB, as well as reconstituted vial contents, were similarly diluted 1:100 in FVIII-deficient plasma prior to assay.

RESULTS

FVIII recovery during 8 hr of CI from PVC bags containing normal saline (method IA) is shown in Table I for the four concentrates, with recovery expressed as percentage of the time 0 level. Both monoclonal products showed a stable recovery of 84–109% of 0 time levels during the 8 hr infusion. Recombinant products 1 and 2 showed an early drop in recovery, reaching a minimum

TABLE I. Factor VIII Recovery During Continuous Infusion Using Method IA (PVC Bags, Normal Saline/No Albumin)

Time (hr)	Product			
	Monoclonal 1[a]	Monoclonal 2	Recombinant 1[b]	Recombinant 2[b]
0	7.9	8.7	5.8	4.5
	(100)	(100)	(100)	(100)
0.25	7.5	8.1	3.3	3.4
	(95)	(93)	(57)	(76)
0.5	6.6	8.5	3.9	3.6
	(84)	(98)	(67)	(80)
1	6.8	8.5	4.0	3.9
	(86)	(98)	(69)	(87)
2	8.1	9.5	5.0	6.4
	(103)	(109)	(86)	(142)
3	7.7	7.4	5.8	7.3
	(97)	(85)	(100)	(162)
4	6.5	9.3	5.3	7.3
	(82)	(107)	(91)	(162)
5	8.5	7.2	6.7	8.5
	(108)	(83)	(116)	(189)
6	7.3	8.2	6.6	8.1
	(92)	(94)	(114)	(180)
7	6.9	7.3	7.0	6.8
	(87)	(84)	(121)	(151)
8	6.9	8.7	7.0	6.5
	(87)	(100)	(121)	(144)

[a]IU/ml (% of time 0 value).
[b]Average of two experiments.

at time 0.25 hr (57% and 76% of time 0, respectively). Recovery reapproximated or exceeded that at time 0 by hour 2 and remained stable for the duration of the 8 hr infusion. When the recombinant products were restudied with the addition of 10 mg/ml of albumin to the NS bags (method IB), no amelioration in the pattern was seen (data not shown).

The use of undiluted product delivered by syringe pump (method IIA), eliminated the early drop in recovery for recombinant products 1 and 2 (Fig. 1A). Stable recovery of 78% to 107% of time 0 level was maintained for the duration of the infusion. Monoclonal product recovery and stability over time were similarly good using either method IA or IIA (Fig. 1B).

Using the initial methodology (method IA), we observed a large discrepancy between the FVIII recovery anticipated from our assay of the reconstituted vial contents and that actually observed in time 0 samples (Table II). This discrepancy was greater for recombinant products than for monoclonal products. We therefore examined the effect of further modifying the delivery systems on this observed discrepancy.

The comparison of FVIII recovery at time 0 with that expected from the assay of vial contents is shown in Figure 2 for the four delivery methods. For all products, recovery was closest to expected (85–98%) using method IIB (undiluted, syringe with albumin). The effect of methodology was greater for recombinant products, for which 49% and 57% initial recovery was noted with method IA, compared to recoveries of 85% and 98% with method IIB.

DISCUSSION

Because of our institutional commitment to the use of both ultrapure FVIII products and CI delivery for the treatment of major hemorrhage and prophylaxis for surgery, it was important to determine the optimal method for integrating both components. Our results with monoclonal products suggest that acceptable monoclonal factor recovery, initially and over 8 hr was achieved in all delivery systems studied, given the ±20% variation allowed by the U.S. Pharmacopaeia [8]. Results were similar for both monoclonal products studied and corroborate the findings of previous studies [6,7].

Recombinant products, on the other hand, showed a strikingly diminished early recovery in the NS/PVC system that was not ameliorated by the addition of 1% albumin to the system. Acceptable and persistently stable recombinant factor recovery was achieved only when factor was delivered by polypropylene syringe without further dilution after recommended reconstitution. In addition to a difference in the composition of the container, these systems differed in the amount of dilution to which the product was subjected. Delivery in bags required a tenfold dilution of the product over that required in the syringes, which delivered the product at the same concen-

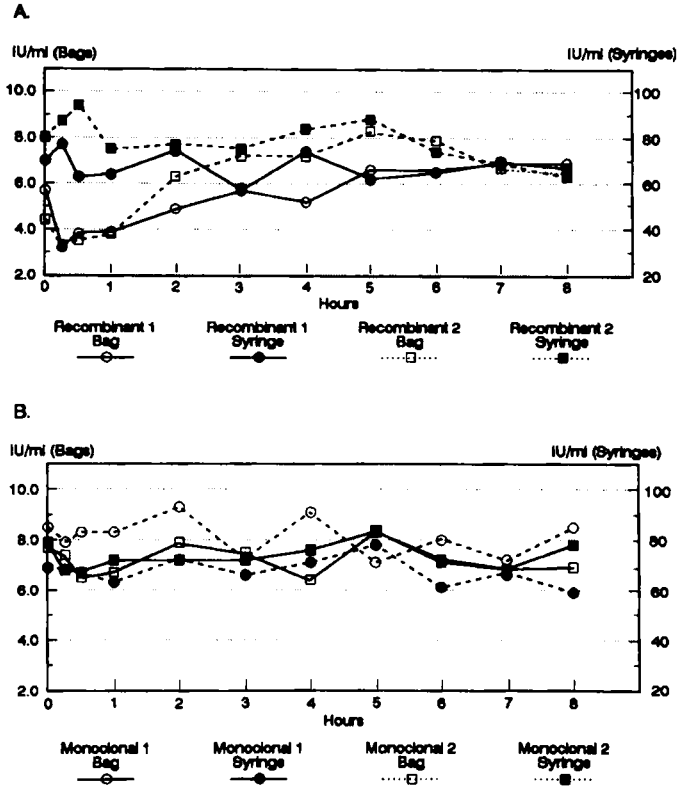

Fig. 1. Recovery and stability of factor VIII, expressed as IU/ml, during continuous delivery using method IA (PVC bags, normal saline/no albumin) and method IIA (polypropylene syringes, undiluted/no albumin). A: Recombinant products. B: Monoclonal products.

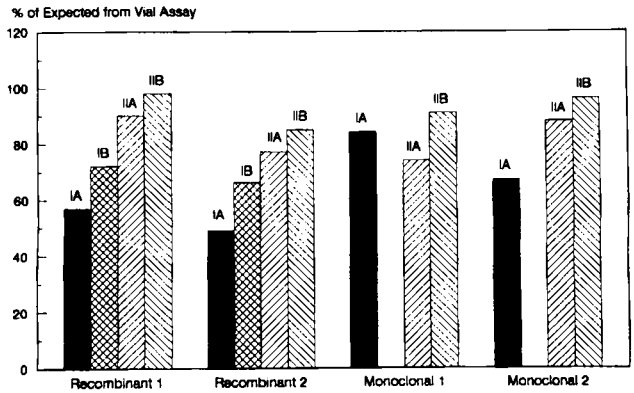

Fig. 2. Recovery of factor VIII at time 0, expressed as percentage of expected from vial assay, for four delivery methods: IA (PVC bags, normal saline/no albumin), IB (PVC bags with albumin), IIA (polypropylene syringes, undiluted/no albumin), and IIB (polypropylene syringes, undiluted/with albumin). (Monoclonal products were not studied using method IB.)

tration used for bolus infusion. The loss of recovery was not a time-dependent function but occurred within the first 1 hr, with subsequent stabilization, suggesting that binding to the delivery system with eventual saturation might have occurred. Schulman et al. [8] previously demonstrated stable recovery of one recombinant product stored in polypropylene after reconstitution according to the manufacturer's recommendations. Since comparable studies in PVC have not been conducted, the relative effects of dilution and plastic surface cannot be ascertained at this time.

These in vitro experiments partially explain our initial clinical observations in those patients who received recombinant, but not monoclonal, products by CI. Interestingly, both Manno et al. [10] and Manco-Johnson et al. [11] have previously reported in vivo recoveries following bolus or CI monoclonal FVIII infusions in children that are 50% of those observed in adults. Increased clearance and plasma volumes in children have been postulated to account for this observation [4,10]. We intend to pursue further in vivo study of this phenomenon in children, now that we have a well-characterized infusion system in which in vitro losses have been minimized.

Using method IIA, we observed initial recoveries for both monoclonal and recombinant products that ranged between 75% and 90% of expected, based on our assay of vial contents. However, because the patient is financially responsible for each unit of FVIII that is administered, the experiment with method IIB was performed in an attempt to further optimize recovery. In a single experiment with each product, recovery was indeed increased by the further addition of human albumin to final concentration of 2% (20 mg/ml). However, further evaluation of this method is necessary before potential gains in cost

TABLE II. Factor VIII Recovery at Time 0 Using Method IA (PVC Bags, Normal Saline/No Albumin)

Product	In-house vial assay (IU/ml)	Expected in bag (IU/ml)	Observed in bag (IU/ml)	Expected-observed (%)
Monoclonal 1	94	9.4	7.9	−16
Monoclonal 2	129	12.9	8.7	−33
Recombinant 1[a]	102	10.2	5.8	−43
Recombinant 2[a]	91	9.1	4.5	−51

[a]Average of two experiments.

effectiveness can be accurately assessed against the cumulative risks of patient exposure to plasma-derived products. When continuous factor infusion is clinically indicated, our institution now delivers ultrapure FVIII products, reconstituted according to manufacturers' recommendations without further saline dilution, using a Medfusion syringe pump. Although no thrombophlebitis has been observed thus far in our patient population, the addition of 1–5 U/ml heparin to the infusion as recommended by Schulman et al. [6] will be considered if such problems are encountered in the future.

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